IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
Before the Board of Patent Appeals and Interferences

In re Patent Application of Atty Dkt. 117-357
C# M#
LINDQVIST et al TC/A.U.: 1639
Serial No. 09/331,808 Examiner: Wessendorf, T.
Filed: January 27, 2000 Date: December 2, 2005
Title: IN VITRO PEPTIDE OR PROTEIN EXPRESSION LIBRARY

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

☐ **Correspondence Address Indication Form Attached.**

☐ **NOTICE OF APPEAL**
Applicant hereby **appeals** to the Board of Patent Appeals and Interferences from the last decision of the Examiner twice/finally rejecting applicant's claim(s). $500.00 (1401)/$250.00 (2401) $

☒ An appeal **BRIEF** is attached in the pending appeal of the above-identified application $500.00 (1402)/$250.00 (2402) $ 250.00

☐ Credit for fees paid in prior appeal without decision on merits -$ ( )

☐ A reply brief is attached. **(no fee)**

☒ Petition is hereby made to extend the current due date so as to cover the filing date of this paper and attachment(s)
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Any future submission requiring an extension of time is hereby stated to include a petition for such time extension. The Commissioner is hereby authorized to charge any deficiency, or credit any overpayment, in the fee(s) filed, or asserted to be filed, or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our **Account No. 14-1140.** A duplicate copy of this sheet is attached.

901 North Glebe Road, 11th Floor
Arlington, Virginia 22203-1808
Telephone: (703) 816-4000
Facsimile: (703) 816-4100
MJW:tat

NIXON & VANDERHYE P.C.
By Atty: Mary J. Wilson, Reg. No. 32,955

12/05/2005 JADD01 00000095 09331808
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Signature: M. J. Wilson

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re Patent Application of — Confirmation No. 2109

LINDQVIST et al — Atty. Ref.: 117-357

Serial No. 09/331,808 — TC/A.U.: 1639

Filed: January 27, 2000 — Examiner: Wessendorf, T.

For: IN VITRO PEPTIDE OR PROTEIN EXPRESSION LIBRARY

* *

December 2, 2005

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APPEAL BRIEF

Sir:

Appellants hereby appeal the final rejection of claims 21, 22, 24-29, 34-36, 39 and 40, in the Office Action dated May 4, 2005, and submit the present Appeal Brief pursuant to 37 CFR § 41.37.

12/05/2005 JADD01 00000095 09331808
02 FC:2402 250.00 OP

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(1) REAL PARTY IN INTEREST

The real party in interest is Isogenica Limited, Stuart House, City Road, Peterborough, United Kingdom PE1 1QF, by way of an Assignment from the inventors to Actinova Limited, 5 Signet Court, Swanns Road, Cambridge CB5 8LA, United Kingdom, recorded in the U.S. Patent and Trademark Office on February 22, 2000, at Reel 010631, Frame 0744, and an Assignment from Actinova Limited to Isogenica Limited, recorded in the U.S. Patent and Trademark Office on April 16, 2001, at Reel 011702, Frame 0648.

(2) RELATED APPEALS AND INTERFERENCES

Appellants, Appellants' legal representative, and the assignee are not aware of any related prior or pending appeals or interferences or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in this appeal.

(3) STATUS OF THE CLAIMS

Claims 21, 22, 24-29, 31, 34-36, 39 and 40 are pending and have been finally rejected.

Claims 5-9 and 11 were amended in the Preliminary Amendment filed June 24, 1999. Claims 1 and 14 were amended and new claims 19 and 20 were added in the Amendment filed January 5, 2001. Original claims 1-18 were cancelled and new claims 21-39 were added in the Amendment Under Rule 116 filed October 9, 2001. Claims 23, 30, 33, 37 and 38 were cancelled, claims 21, 22, 24-29, 31, 32, 34-36 and 39 were amended and new claim 40 was added in the Amendment filed May 5, 2003. Claims 19 and 20 were cancelled and claims 21, 29, 31 and 34-36 were amended in the Amendment Under Rule 116 filed July 22, 2004. Claim 31 was amended and claim 32 was cancelled in the Amendment filed December 20, 2004. Claim 31 was cancelled in the Amendment Under Rule 116 filed September 6, 2005.

Claims 21, 22, 24-29, 34-36, 39 and 40 are the subject of the present appeal. A copy of claims 21, 22, 24-29, 34-36, 39 and 40 is attached as a Claims Appendix, pursuant to Rule 41.37(c)(1)(viii).

(4) STATUS OF THE AMENDMENTS

The Amendment Under Rule 116 filed October 9, 2001, responsive to the final Office Action dated April 9, 2001, has been entered

The Amendment Under Rule 116 filed July 22, 2004, responsive to the final Office Action dated October 20, 2003, has been entered

An Amendment Under Rule 116 was filed September 6, 2005, in response to the final Office Action dated May 4, 2005. In the Advisory Action dated October 14, 2005, the Examiner indicated that the request for reconsideration presented in that Amendment had been considered. It appears that the Examiner may have overlooked the fact that claim 31 was cancelled in the September 6 Amendment as the Advisory Action does not include a clear statement, positive or negative, as regards entry of proposed amendments. Under "status of the claims", however, claim 31 is indicated as being rejected.

(5) SUMMARY OF CLAIMED SUBJECT MATTER

The present invention, as claimed in claim 21, and claims 22, 24-29, 34, 35, 39 and 40 which depend therefrom, relates to a method of producing a peptide or protein expression library which displays a population of peptides or proteins. The peptides or proteins are specifically associated with the DNA encoding them through covalent binding of the peptides or proteins to the encoding DNA. The method comprises at least the following steps:

1) preparing a genetic library of a population of DNA molecules, each DNA molecule comprising:

(a) a nucleotide sequence encoding a binding moiety comprising an amino acid sequence which is a *cis*-acting DNA binding protein which binds specifically to the DNA encoding sequence through covalent binding of the amino acid sequence to DNA, and

(b) a nucleotide sequence encoding a display moiety comprising an amino acid sequence for display, and wherein the display moiety comprises at least one site of attachment for the binding moiety, and

2) expressing the genetic library thus formed.

The population of peptides or proteins thus produced is each specifically associated with the DNA encoding sequence through covalent binding. Support for this aspect of

the invention can be found throughout the application, with particular attention being directed to page 5, line 32 to page 6, line 12, page 7, lines 27-30, and original claim 1.

The present invention, as claimed in claim 36, relates to a method of assaying for the presence of a target molecule in a sample. This method comprises:

(a) contacting the sample with a molecular probe comprising:

(i) a peptide or protein target-binding moiety that selectively binds to the target molecule, wherein the target-binding moiety is covalently bound to DNA encoding the target-binding moiety, and

(ii) a reporter moiety

wherein the contacting is effected under conditions such that the target-binding moiety can bind target molecule present in the sample selectively; and

(b) detecting the presence of reporter moiety bound to the target-bound molecular probe. Support for this aspect of the invention can be found, for example, at page 39, lines 3-13, and in original claim 17.

(6) GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The following grounds of rejection are presented for review:

Whether the invention of claims 21, 22, 24, 26-28, 34-36 and 39 lacks novelty under 35 USC 102(e) over Maruyama et al (USP 5,627,024).

Whether claims 21, 22, 24-29, 34-36, 39 and 40 would have been obvious under 35 USC 103(a) over Maruyama et al in view of Liu et al (Virology 216:158-164 (1996)).

(7) ARGUMENT

NOVELTY (35 USC 102(e))

The subject matter of claims 21, 22, 24, 26-28, 34-36 and 39 is novel. Accordingly, reversal of the rejection under 35 USC 102(e) based on Maruyama et al is respectfully requested.

The instant invention relates to a method of producing a peptide or protein expression library that displays a population of peptides or proteins. The peptides or proteins are specifically associated with the DNA encoding them through covalent binding of the peptides or proteins to the encoding DNA. The method comprises: i) preparing a genetic library of a population of DNA molecules, each DNA molecule comprising a nucleotide sequence encoding a binding moiety comprising an amino acid sequence that is a *cis*-acting DNA binding protein that binds specifically to the DNA encoding sequence through covalent binding of the amino acid sequence to DNA, and a nucleotide sequence encoding a display moiety, and ii) expressing the genetic library thus formed whereby the population of peptides or proteins is produced each specifically associated with the DNA encoding sequence through covalent binding. (At page 7, lines 27-30 of the subject application, *cis*-acting proteins are defined as proteins that bind covalently to the same DNA sequence that served as the template for their synthesis.) The protein-DNA complexes can be used for the affinity selection of target-binding polypeptides. The covalent association and co-selection of

the DNA that encodes the target-binding polypeptides enables the recovery and identification of the relevant sequences (a specific embodiment of the invention (relating to the *cis*-acting protein P2-A) is depicted in Figure 1 which is provided merely for purposes of exemplification and to facilitate understanding – this Figure was first submitted with the Amendment filed July 22, 2004 (see Exhibit A)).

The method of Maruyama et al involves the use of lambdoid phages as alternatives to the use of filamentous phages for the phage display of polypeptides. The Maruyama et al method is conceptually identical to phage display as practiced using the filamentous phage M13. It involves the construction of a plurality of modified phage genomes in which the gene for one of the phage coat or tail proteins is fused in frame with a population of genes for display. Upon insertion of the pool of genomes into an appropriate *E.coli* host (transformation), the phage genome replicates and results in the production of progeny phage in which the phage head structure contains the modified genome and the tail structure displays the polypeptide encoded by the fused gene within the modified genome (for ease of understanding, lamboid display is illustrated in Figure 7 - Figure 7 was submitted with the Amendment filed September 6, 2005 (see Exhibit B), and was supplementary to the Figures submitted with the Amendment filed July 22, 2004).

In rejecting the claims as anticipated, the Examiner refers to the following sections of Murayama et al:

- Column 16, lines 10-45, where, according to the Examiner "the formula of a fusion of the polypeptide to its DNA encoding sequence" is disclosed. This is represented by the formula NH_2-O-U-V-COOH, where O represents an amino acid residue sequence defining a lambdoid matrix anchor polypeptide (a portion of a phage coat protein or tail protein), U represents a linker polypeptide and V represents an amino acid residue.
- Column 16, line 20, where it is stated that "a preferred polypeptide comprises a preselected polypeptide operatively linked at is amino-terminus to the lambdoid matrix anchor polypeptide".
- Column 16, line 40, where it is stated that "'operatively linked' means that the polypeptide fragments ... have been covalently joined ...".
- Column 2, lines 29-34, where it is stated that the "multimeric polypeptides and the genes which encode the polypeptides are thus physically linked during the assembly stage of the phage replication cycle".
- Column 8, lines 62-63, where it is disclosed that the lamdoid phage particles are about half protein and half DNA.

The Examiner's assertions to the contrary, nothing in Maruyama et al, including the above-referenced portions, teaches the existence of a direct **covalent** interaction between the displayed polypeptide (e.g., the product of the formula F1 as stated in column 16, lines 10-20) and the DNA that encoded it. Indeed, the disclosures of Maruyama et al suggest that the only components of the method that are covalently linked are the displayed polypeptide and the lambdoid matrix anchor polypeptide itself (i.e., NH_2-O-U-V-COOH) which are fused via a linker peptide through standard peptide bonds. Nowhere does it state that there is any "fusion" between the product of formula F1 and "its DNA encoding sequence".

Furthermore, the Murayama et al method is entirely reliant on the assembly of phage particles during the phage replication cycle whereas, for the practice of covalent

display, the use of phage particles, mammalian viruses, eukaryotic cells, bacteria or any other biological entities, are not required.

Finally, the DNA:protein complexes that are formed in covalent display are not nearly in a ratio of 50:50, they are in fact closer to a ratio of 10:1. This is because, in addition to the encoding DNA, there is just one protein associated with it.

The Murayama et al technique differs significantly from the covalent display technique of the present invention. Notably, the Murayama et al method requires the transformation of *E.coli* with the DNA that comprises the modified genome. This then facilitates the production of the lambda phages that display the polypeptides of interest. The physical linkage between the expressed polypeptide and its encoding DNA is, therefore, not through a direct covalent bond between the expressed polypeptide and its encoding DNA, as it is with covalent display, rather it is through the *in vivo* assembly of a viable phage particle that has the encoding DNA packaged within the head of the particle and the displayed polypeptide attached to the tail protein.

The *cis*-activity obviates the need to perform the transformation step that is common to both M13 and lambdoid phage display. The *cis*-activity enables the linkage between the gene and gene product to be formed without confining individual genes within a bacterial (or any other) cell prior to their transcription and translation. The DNA constructs produced by the Murayama et al method cannot form a physical

linkage between a gene and its expressed polypeptide unless the *in vivo* phage assembly process occurs. Such phage assembly is not required in the covalent display method.

No *cis*-acting DNA binding proteins (as noted above, defined for covalent display as being proteins that bind covalently to the same DNA molecule that served as the template for their synthesis – see page 7, lines 27-30 of the subject application) are disclosed or contemplated by Murayama et al.

The mechanisms by which a library of peptides that are linked to their encoding nucleic acids are achieved are thus very different as between the Murayama et al and covalent display methods. Therefore, Appellants' invention is not taught by Maruyama et al.

In view of the above, reversal of the rejection is requested.

OBVIOUSNESS (35 USC 103(a))

The subjected matter of claims 21, 22, 24-29, 34-36, 39 and 40 would not have been obvious. Accordingly, reversal of the rejection under 35 USC 103(a) based on Maruyama et al in view of Lui et al is respectfully requested.

The comments set forth above in connection with the rejection under 35 USC 102(e) make clear the fundamental differences between Maruyama et al and the

covalent display approach of the present invention. Liu et al adds noting to Maruyama et al that would have rendered the present invention obvious.

Lui et al relates to the construction of individual plasmids containing P2-A wild type, P2-A (Y450D), P2-A (Y454F) or P2-A (Y450D and Y454F). Each P2-A variant was cloned in-frame with a His tag. Proteins were expressed in *E.coli* to produce inclusion bodies. The inclusion bodies were isolated and the P2-A variants purified using the His tags to which they were fused. Biochemical studies were then conducted on purified proteins to understand the catalytic mechanism of P2-A (for ease of understanding, see attached Figure 2, originally submitted with the Amendment filed July 22, 2004 (see Exhibit A)).

In Lui et al, there is no disclosure of the recovery of covalent protein:DNA complexes following expression in *E.coli*, only the expressed and purified protein is recovered for biochemical analyses (cleavage of single stranded oligouncleotides containing the ori sequence). There is no disclosure of the formation of a library consisting of P2-A fused to a plurality of peptides. There is no suggestion in Lui et al that the *cis* property of P2A could be exploited for a library screening method.

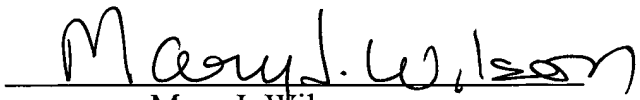
Given the unrelatedness of Maruyama et al and Lui et al, there is nothing in their respective teachings that would have suggested their combination. Further, even if the references had been combined (which they would not have been), that

combination would not have rendered the claimed invention obvious. Accordingly, reversal of the rejection is requested.

In view of the foregoing, it will be clear that the claims are in condition for allowance and reversal of the final rejection is, therefore, requested.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: 
Mary J. Wilson
Reg. No. 32,955

MJW:tat
1100 North Glebe Road, 8th Floor
Arlington, VA 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

(8) CLAIMS APPENDIX

21. A method of producing a peptide or protein expression library which displays a population of peptides or proteins, wherein the peptides or proteins are specifically associated with the DNA encoding them through covalent binding of the peptides or proteins to the encoding DNA, said method comprising at least the following steps:

1) preparing a genetic library of a population of DNA molecules, each DNA molecule comprising:

(a) a nucleotide sequence encoding a binding moiety comprising an amino acid sequence which is a *cis*-acting DNA binding protein which binds specifically to the DNA encoding sequence through covalent binding of the amino acid sequence to DNA, and

(b) a nucleotide sequence encoding a display moiety comprising an amino acid sequence for display, and wherein the display moiety comprises at least one site of attachment for the binding moiety, and

2) expressing the genetic library thus formed whereby the population of peptides or proteins is produced each specifically associated with the DNA encoding sequence through covalent binding.

22. The method as claimed in claim 21 wherein expression of the genetic library is performed *in vivo* with at least one copy of a single library member expressed per host cell or organism.

24. The method as claimed in claim 21 wherein expression of the genetic library is performed *in vitro*.

25. The method as claimed in claim 21 wherein said *cis*-acting protein is the P2 A protein.

26. The method as claimed in claim 24 wherein said expression is performed in the presence of a mis-match oligonucleotide which hybridizes to the DNA adjacent to the attachment site on both sides but that does not hybridize to the attachment site.

27. The method as claimed in claim 21 wherein said amino acid sequence for display is up to 40 amino acid residues.

28. The method as claimed in claim 21 wherein said amino acid sequence for display is generated by, or comprises DNA fragments from, cloning.

29. A method as claimed in claim 21 wherein said binding moiety is P2A modified by replacement of tyrosine at amino acid position 450 with phenylalanine.

34. A method of identifying a specific target-binding peptide or protein, said method comprising:

a) contacting a peptide expression library produced according to the method of claim 21 with a target molecule,

b) selecting and isolating a library member that binds to said target molecule, and

c) isolating from said library member the peptide or protein that is bound to said target molecule.

35. The method as claimed in claim 34 further comprising isolating from said library member the DNA sequence encoding the peptide or protein that binds specifically to said target molecule.

36. A method of assaying for the presence of a target molecule in a sample, said method comprising

(a) contacting said sample with a molecular probe comprising

(i) a peptide or protein target-binding moiety that selectively binds to said target molecule, wherein said target-binding moiety is covalently bound to DNA encoding said target-binding moiety and

(ii) a reporter moiety

wherein said contacting is effected under conditions such that said target-binding moiety can bind target molecule present in said sample selectively; and

(b) detecting the presence of reporter moiety bound to said target-bound molecular probe.

39. The method according to claim 21, wherein said nucleic acid encoding said amino acid sequence for display is generated by amplification by PCR.

40. The method according to claim 21 wherein the cis-acting protein is ϕX174.

(9) EVIDENCE APPENDIX

Attached

Exhibit A	Figures 1 and 2 submitted with Amendment Under Rule 116 filed July 22, 2004, with Request for Continued Examination; entry acknowledged by Examiner in Office Action dated August 24, 2004
Exhibit B	Figure 7 submitted with request for consideration in Amendment Under Rule 116 filed September 6, 2005; consideration of request for reconsideration acknowledged by Examiner in Advisory Action dated October 14, 2005

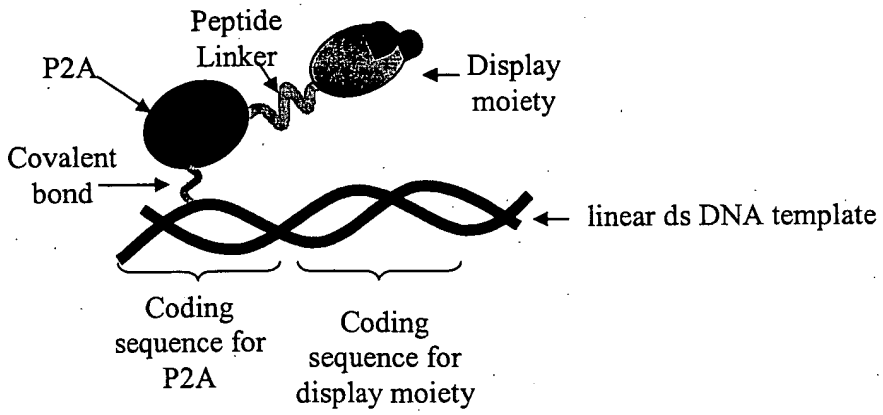


Figure 1. Covalent display
Double stranded DNA containing the coding sequence for P2-A and the coding sequences for a diverse population of polypeptides is transcribed and translated *in vitro* and due to the cis-activity of P2-A, the expressed polypeptides spontaneously and covalently associate with their own encoding DNA molecules through the interaction between P2-A and its recognition sequence which is contained within the P2-A gene. The covalent Protein-DNA complexes are then used in affinity selection protocols against a given target in order to identify individual genes that encode ligands to the target.

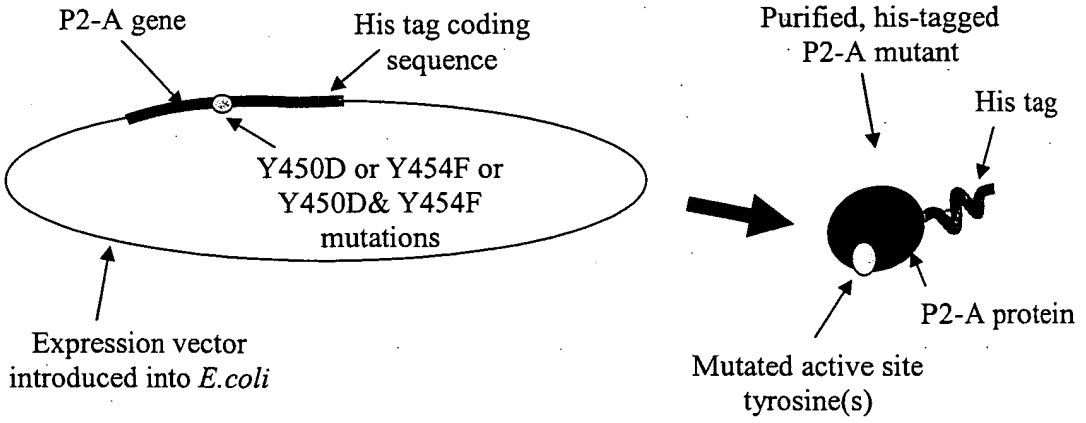


Figure 2. Liu's disclosure
P2-A wild type sequence, or P2-A genes in which either the putative active site tyr450, the putative active site tyr454 or both putative active site tyrosines were mutated, were cloned in frame with a hexa-histidine tag. The expressed polypeptides from these constructs formed inclusion bodies from which purified P2-A variants were isolated and used in biochemical analyses of the catalytic mechanism of P2-A. No attempt to isolate covalent complexes consisting of the P2-A gene and the expressed P2-A protein was made. No diverse population of P2-A-fused binding domains was made. No indication was made that the cis property of P2-A could be exploited for the establishment of a library screening method.

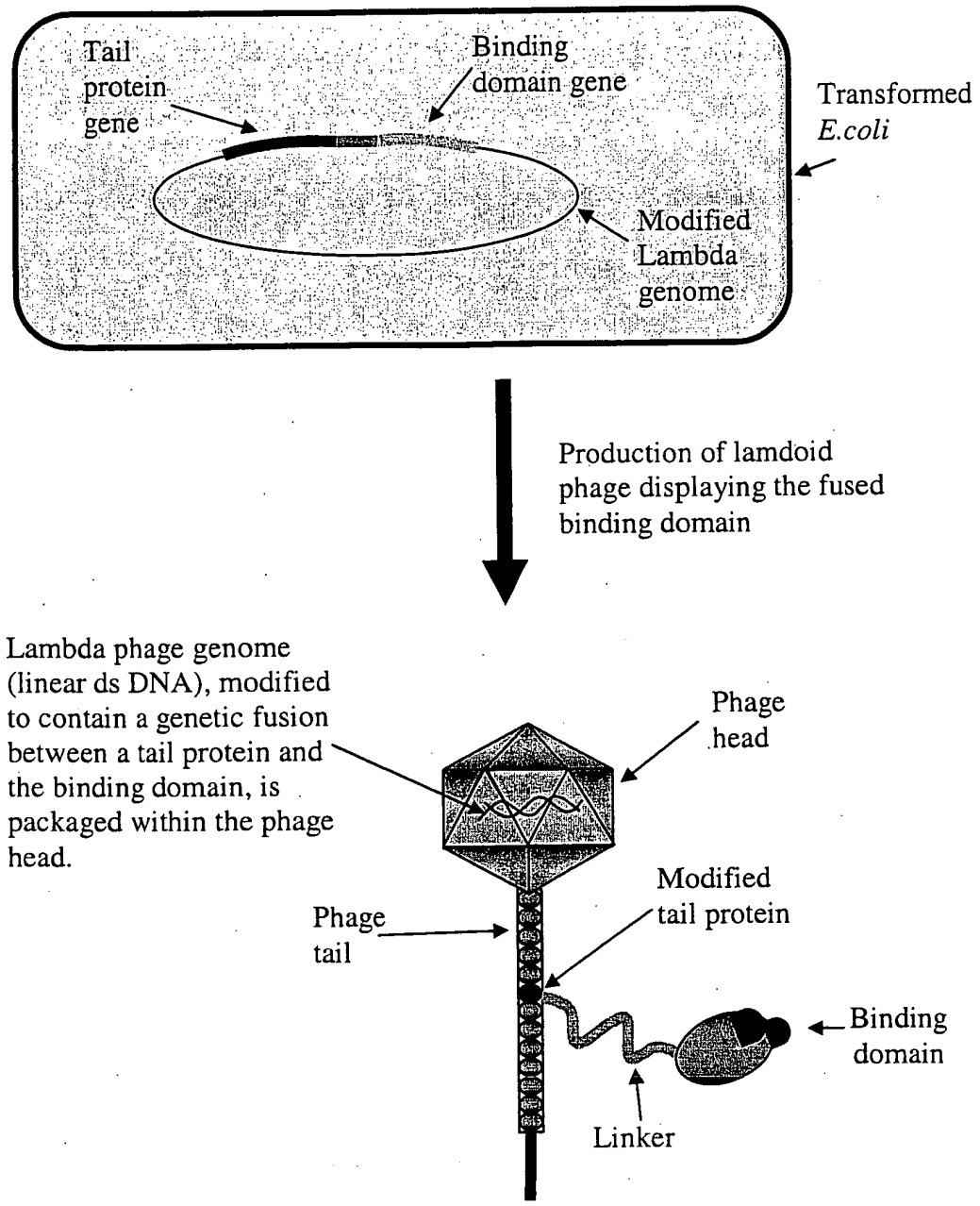


Figure 7. Lambdoid display
The coding sequences for a diverse population of polypeptides are fused in frame with the gene encoding the tail protein of phage lambda. Upon production on new phages, the modified genome is packaged into the phage head while the displayed polypeptide is fused to a tail protein in such a way that it is accessible for interaction with external binding partners. The phages so generated are then used in affinity selection protocols against a given target in order to identify individual genes that encode ligands to the target.

(9) RELATED PROCEEDINGS APPENDIX

NONE